Data Evaluation Report on the Chronic Toxicity of Mesotrione to Mysid, *Americamysis bahia*

MRID 50096301

Data Requirement:

EPA DP Barcode: 437913
EPA MRID: 50096301
EPA Guideline: 850.1350

Test Material: Mesotrione
Common name: Mesotrione technical
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: 104206-82-8
Synonyms: Not reported

Purity (%): 84.6

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Staff Scientist, CDM/CSS-Dynamac JV
Signature: Rebecca L. Bryan
Date: 8/1/2018

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Date: 10-2-2020

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Date Evaluation Completed: 10-2-2020

CITATION

Viveiros, A. 2016. Mesotrione - Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*). Unpublished study performed by Smithers Viscient Laboratories, Wareham, Massachusetts. Laboratory Study No. 1781.7122. Study sponsored by Syngenta Crop Protection, LLC, Greensboro, North Carolina. Study initiated January 7, 2016 and completed September 16, 2016.

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EXECUTIVE SUMMARY

The 28-day chronic toxicity of mesotrione to mysids (*Americamysis bahia*) was studied under flow-through conditions. Mysids were exposed at nominal concentrations of 0 (negative control), 3.1, 6.3, 13, 25, and 50 µg ai/L. The mean-measured concentrations were <0.25 (<LOQ, control), 3.1, 6.8, 12, 26, and 50 µg ai/L, respectively. No significant treatment-related effects were observed for F_0 survival (pre- and post-pairing), F_0 dry weight (male and female), F_0 length (male and female), F_1 survival, or time to first brood. The only affected endpoint was the number of offspring per female. The potential effect was not statistically significant but rather judged on multiple lines of evidence as discussed in the Reviewer's Comments section based on potential biological significance of the magnitude of the reductions in the number of offspring (14% and 25%) at the two highest test concentrations.

This study is scientifically sound and is classified as **Acceptable**.

Results Synopsis

NOAEC = 12 µg ai/L
LOAEC = 26 µg ai/L based on reduced number of offspring per female

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in the U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OCSPP draft Guideline 850.1350 Mysid Chronic Toxicity Test (1996). A deviation from OCSPP 850.1350 was observed.

1. Physiochemical properties of mesotrione technical were not reported.

This deviation does not impact the acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided. This study was conducted in accordance with U.S. EPA GLP Standards as published in 40 CFR Part 160 with the following exception: routine food and water contaminant screening analyses were performed by GeoLabs, Inc. (Braintree, Massachusetts) using standard U.S. EPA analytical methods. This exception had no impact on the study results.

A. MATERIALS

1. Test Material: Mesotrione

Description: Not reported

Lot No./Batch No.: 675385 (Batch No.)

Purity: 84.6%

Stability of compound under test conditions: The reported time-weighted average concentrations were 94-110% of nominal with coefficient of variance ranging from 8.8 to 16%. The reviewer's mean-measured concentrations yielded CVs of 12 to 15%.

Storage conditions of test chemicals: Room temperature

Physicochemical properties of mesotrione

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism

Species: Mysid (*Americamysis bahia*)
Age class/Age: ≤24 hours old
Source: In-house laboratory culture (brood stock from MBL Aquaculture, Sarasota, Florida).

B. STUDY DESIGN

1. Experimental Conditions

a. Range-finding study

The concentrations for the definitive study were selected based on the results from 28-day range-finding toxicity studies and consultation with the study sponsor. The flow-through range-finding study was conducted at nominal concentrations of 10, 31, 100, 310, and 1000 µg ai/L with a negative control, using 40 mysids per treatment group (20 mysids per replicate). Male survival averaged 95, 84, 70, 0, and 0% in the nominal 10, 31, 100, 310, and 1000 µg ai/L groups, compared to 93% in the control. Female survival averaged 86, 100, 29, 0, and 0% in the nominal 10, 31, 100, 310, and 1000 µg ai/L groups, compared to 100% in the control. Mean post-pairing survival was 92, 90, 41, 0, and 0% in the 10, 31, 100, 310, and 1000 µg ai/L groups, compared to 97% in the control. The mean 28-day survival was 74, 70, 29, 0, and 0% in the 10, 31, 100, 310, and 1000 µg ai/L groups, compared to 76% in the control. Survival was significantly different from the control in the ≥100 µg ai/L groups compared to the control.

For males, the mean total lengths were 7.38, 7.44, and 7.59 mm in the nominal 10, 31, and 100 µg ai/L groups, compared to 7.30 mm in the negative control. The mean dry weights for males were 0.75, 0.82, and 0.81 mg in the 10, 31, and 100 µg ai/L groups, compared to 0.79 mg in the negative control. For females, the mean total lengths were 7.55, 7.68, and 7.76 mm in the 10, 31, and 100 µg ai/L groups, compared to 7.74 mm in the negative control. The mean dry weights for females were 1.03, 1.13, and 1.20 mg in the 10, 31, and 100 µg ai/L groups, compared to 1.15 mg in the negative control.

The time to maturation was 9, 10, and 11 days in the 10, 31, and 100 µg ai/L groups, compared to 9 days in the negative control. The time to first brood release was 21, 21, and 19 days in the 10, 31, and 100 µg ai/L groups, compared to 19 days in the negative control. The mean offspring per female were 13.8, 11.5, and 3.6 in the 10, 31, and 100 µg ai/L groups, compared to 16.7 in the negative control. The mean offspring per female were significantly different in the 100 µg ai/L group compared to the control. The mean percentage of reproductively active females were 100, 90, and 45% in the 10, 31, and 100 µg ai/L groups, compared to 100% in the negative control. The F_1 survival at 96 hours was 94, 75, and 95% in the 10, 31, and 100 µg ai/L groups, compared to 100% in the negative control.

b. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
<u>Acclimation</u>		
Period	Continuous culture	*EPA recommends within a 24–h period, changes in temperature should be ≤ 1°C and changes in salinity be ≤ 5%.*
Conditions	Same as test	*Mysids should be cultured and tested in dilution water from the same origin.*
Feeding	Not reported	*Mysids should be fed daily during testing, as necessary to support survival, growth and reproduction. Artemia spp. (48–h–old nauplii) is recommended.*
Health	The culture organisms did not show any sign of sickness, disease, injuries, or abnormalities from the day of receipt to the day of exposure initiation.	
Duration of the test	28 days	
Time of pairing	Pairing on Day 13	*The recommended test duration is 28 days Pairing occurs when mysids reach sexual maturity Length should be measured at the time of sexual discernment.*
<u>Test condition</u>		The function of the diluter was monitored daily and a visual check of the system's operation was performed twice daily. The exposure system was functioning properly for 12 days prior to exposure initiation. The flow rate accuracy was 5% for each replicate.
Flow-through	Flow-through	*EPA recommends flow-through systems.*
Type of dilution system - for flow-through method	Proportional intermittent-flow diluter, flow rate of 7.7 volume additions per day	*For flow-through tests, the flow rate should be ≥ 5 vol/24 hours; diluter systems should be calibrated before each test and checked twice daily during the test. Flow rates should not vary by >10% within and between replicates.*
		A reproducible supply of toxicant is recommended.
Aeration	No aeration during testing.	
		EPA recommends if aeration is needed to achieve DO level, it should be done before the addition of the test substance, and all treatment and control chambers should be given the same aeration treatment.

Parameter	Details	Remarks *Criteria*
<u>Test Vessels</u> Material	Glass aquaria	*EPA recommends materials/equipment that have minimal sorption of test chemicals from dilution water and contain no substances that can be leached and affect test results.* *Test vessels should be loosely covered.* *EPA recommends that mysids be held in retention chambers within test chambers to facilitate observations and eliminate loss through outflow water; netting material should be of appropriate mesh size.*
Size	Prior to pairing: 30 x 15 x 20 cm with a 10-cm side drain (4.5 L volume) After pairing: 30 x 15 x 20 cm with a 10-cm side drain (4.5 L volume)	
Fill volume	Prior to pairing: *ca*. 785 mL After pairing: *ca*. 250 mL	
<u>Retention Chambers</u> Material	Glass petri dish with Nitex® screen collar (14 cm high, 350-µm mesh size)	
Size	Prior to pairing: 10-cm diameter, 2-cm deep After pairing: 6-cm diameter, 1.5 cm deep	
Source of dilution water	Dilution water consisted of diluted, filtered natural seawater. The seawater was pumped from the Cape Cod Canal (Bourne, MA) from *ca*. 1-4 m offshore at a depth of ca. 0.5 m. In the laboratory, the seawater was diluted with laboratory well water and filtered (20-, 5-, and 1-µm) prior to use.	Representative samples of the dilution water source were analyzed periodically for the presence of pesticides, PCBs, and toxic metals by GeoLabs, Inc. (Braintree, Massachusetts). None of these compounds have been detected at concentrations that are considered toxic in any samples analyzed.
<u>Quality of dilution water</u>		*Recommended source of dilution water is natural or artificial seawater that mysids will survive and successfully reproduce in for the duration of the holding, acclimating, and testing periods without showing signs of stress. Prior to use, natural seawater should be filtered through a >20 µm filter. Deionized water with a conductivity <0.1 mS/m (1µohm/cm) at 12°C is acceptable for making artificial seawater. If ground or surface water was used to make deionized water, then conductivity and TOC (or COD) should be measured on each batch.*
Salinity	20-23‰	
pH	7.6-7.8	
Specific Conductivity	Not reported	
TOC	1.2 and 0.86 mg/L for April and May 2016, respectively	
COD	Not reported	

Parameter	Details	Remarks *Criteria*
Water quality during testing pH Dissolved oxygen Temperature Salinity Other measurements Photoperiod Light intensity Intervals of water quality measurement	 7.3-7.9 5.5-7.3 mg/L (76 to 100% saturation) Daily: 25-26°C Continuous: 25-27°C 20-22‰ 16 hour light/8 hour dark with 30-minute transition period 290 to 620 lux Temperature, dissolved oxygen, pH, and salinity were measured in each aquarium at test initiation and daily in alternating replicates thereafter. Additionally, temperature was continuously measured in one control replicate.	 *EPA Recommendations:* *Dissolved Oxygen: 60-105% saturation* *Temperature: 25 ± 2°C* *Salinity: 20±3 ‰.* *Photoperiod: 14–h light and 10–h dark, with a 15 to 30 min transition period.* *pH, DO, temperature, and salinity should be measured weekly in each test chamber.*
Number of replicates/groups: Pre-pairing Negative control Solvent control Treatments Post-pairing Negative control Solvent control Treatments	 4 N/A 4 4 N/A 4	 *A minimum of two replicates per treatment level and control.*
Number of organisms per replicate /groups Pre-pairing Negative control Solvent control Treatments Post-pairing Negative control Solvent control Treatments	 20 N/A 20 10 N/A 10	After pairing, each replicate contained 5 pairs (1 male:1 female) of mysids. For the F1 generation, each replicate compartment contained 10 offspring. *A minimum of 40 mysids per concentration should be exposed, and physically separated into replicate groups of no more than 8 individuals when most of the mysids reach sexual maturity (usually 10–14 days after the beginning of the test).* *Test organisms should be impartially distributed to show no significant bias from the distributions.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate	Biomass loading did not exceed 0.0045 g/L under the exposure's flow-through conditions.	Based on the typical average wet weight of 0.0045 g for adult mysid. *The number of mysids placed in a test solution should not be so great as to affect results of the test. The loading should not cause DO to fall below the recommended levels.*
<u>Test concentrations</u> Nominal Study author calculated time-weighted average Reviewer calculated mean-measured	0 (negative control), 3.1, 6.3, 13, 25, and 50 µg ai/L <0.25 (<LOQ, control), 3.1, 6.8, 12, 27, and 49 µg ai/L <0.25 (<LOQ, control), 3.1, 6.8, 12, 26, and 50 µg ai/L	The % CV range was 8.8 to 16 and 12 to 15% based on the reported TWA and reviewer-calculated mean-measured concentrations, respectively. *EPA recommends a minimum of 5 test concentrations, in geometric series with a ratio of 1.5 to 2, plus a control/solvent control.* *Test concentration must be measured at each test concentration level at test initiation and on days 7, 14, 21 and 28, and after every malfunction in the appropriate chamber.* *Measured concentration of test substance should not vary >20% among replicate test chambers.*
Solvent	None	 *The solvent should not exceed 0.1 ml/L.*
Feeding	Mysids were fed live brine shrimp (*Artemia salina*) nauplii, ≤48 hrs old, twice daily. At least one of these feedings was with brine shrimp nauplii enriched with Selco® (a supplemental substance high in saturated fatty acids). <u>F_0-generation</u> Days 0-3: 90 nauplii/mysid Days 4-6: 135 nauplii/mysid Days 7-9: 180 nauplii/mysid Days 10-12: 225 nauplii/mysid Days 13+ (pairing chambers): 450 nauplii/mysid Days 13+ (retention chambers): 3600 nauplii/chamber <u>F_1-generation</u> 90 nauplii/mysid	Excess food and organic debris were siphoned from the test chambers daily. *Mysids should be fed daily during testing, as necessary to support survival, growth and reproduction. Artemia spp. (48–h–old nauplii) is recommended.*
G_2 exposure	96 hours	

Parameter	Details	Remarks *Criteria*
Recovery of chemical Frequency of determination Level of quantitation Level of detection	94-110% of nominal 0, 7, 13, 14, 21, and 28 days 0.25 µg ai/L Not reported	Recoveries based on reported TWA concentrations (%CV=8.8-16%). Samples were collected on day 13 due to a Day 12 exposure system malfunction which left the exposure vessels in a static state overnight. The QC sample recoveries for method validation were 81.9-116% of nominal.
Positive control	None reported	
Other parameters	None	

2. Observations

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	- F_0 survival and sublethal effects - Day of first brood - Offspring per day and percent of females producing young - F_0 total length and dry weight - F_1 offspring survival (G2) and sublethal effects	*Recommended parameters measured include:* *- survival of first generation mysids (F_0) pre- and post-pairing;* *- number of offspring produced per female;* *- time to sexual discernment;* *- time to first brood;* *- body length and dry weight of females, and body length and dry weight of males (F_0) (body length is measured by total midline body length, from the anterior tip of the carapace to the posterior margin of the uropod)* *- survival of offspring (F_1);* *- incidence and description of morphological abnormalities and behavioral effects;* *- observations of other effects or clinical signs.*
Observation intervals	F_0 Survival and clinical signs-Daily Reproduction- Daily F_0 Length and Dry Weight- Day 28 F_1 - Daily up to 96 hours	*Mortality and other observations should be recorded at test initiation and on day 7, 14, 21 and 28. The number of male and female mysids in each test chamber should be recorded at the time when sexual characteristics become discernible. Observation of male and female body lengths and dry weights should be conducted on day 28 of the test. As offspring are produced, the young should be counted and separated into retention chambers at the same test substance concentration as the chambers where they originated.*
Were raw data included?	Yes	

Parameter	Details	Remarks
		Criteria
Other observations	None	

II. RESULTS AND DISCUSSION

A. MORTALITY AND SUB-LETHAL EFFECTS

At 28 days, mean F_0 survival was 84, 73, 82, 69, and 74% in the time-weighted average concentrations of 3.1, 6.8, 12, 27, and 49 µg ai/L groups, respectively, compared to 75% in the negative control. Based on F_0 survival at 28 days, the NOAEC was 49 µg ai/L and the LOAEC was >49 µg ai/L.

No behavioral abnormalities were observed during the study.

The male body lengths at 28 days averaged 7.54, 7.54, 7.42, 7.58, and 7.44 mm in the time-weighted average concentrations of 3.1, 6.8, 12, 27, and 49 µg ai/L groups, respectively, compared to 7.68 mm in the negative control. The female body lengths at 28 days averaged 7.73, 7.63, 7.65, 7.64, and 7.61 mm in the time-weighted average concentrations of 3.1, 6.8, 12, 27, and 49 µg ai/L groups, respectively, compared to 7.85 mm in the negative control. Based on length, the NOAEC was 49 µg ai/L and the LOAEC was >49 µg ai/L.

The male dry weights at 28 days averaged 0.86, 0.87, 0.89, 0.82, and 0.90 mg in the time-weighted average concentrations of 3.1, 6.8, 12, 27, and 49 µg ai/L groups, respectively, compared to 0.88 mg in the negative control. The female dry weights at 28 days averaged 1.19, 1.24, 1.15, 1.19, and 1.10 mg in the time-weighted average concentrations of 3.1, 6.8, 12, 27, and 49 µg ai/L groups, respectively, compared to 1.18 mg in the negative control. Based on dry weight, the NOAEC was 49 µg ai/L and the LOAEC was >49 µg ai/L.

Table 3. Effect of Mesotrione on Survival and Growth of Adult Mysid *sp.* [A]

Time-Weighted Average (Nominal) Concentration (µg ai/L)	Percent Survival (mean)				Day 28 Growth (mean ± SD)			
	Male	Female	Days 13 to 28	Day 28	Total Body Length (mm)		Dry Body Weight (mg)	
					Male	Female	Male	Female
Negative Control (<LOQ)	81	87	85	75	7.68±0.13	7.85±0.12	0.88±0.08	1.18±0.16
3.1 (3.1)	88	93	91	84	7.54±0.06	7.73±0.18	0.86±0.04	1.19±0.09
6.8 (6.3)	76	91	83	73	7.54±0.09	7.63±0.12	0.87±0.03	1.24±0.07
12 (13)	88	96	93	82	7.42±0.06 [B]	7.65±0.04	0.89±0.09	1.15±0.14
27 (25)	75	83	80	69	7.58±0.27	7.64±0.22	0.82±0.07	1.19±0.04
49 (50)	81	95	87	74	7.44±0.12	7.61±0.18	0.90±0.07	1.10±0.06
NOAEC, µg ai/L	49				49	49	49	49
LOAEC, µg ai/L	>49				>49	>49	>49	>49

[A] Data were obtained from study report Tables 3, 5 and 6 on pages 33, 35, and 36, respectively.
[B] Significantly reduced compared to the control (Dunnett's Multiple Comparison Test). Due to a lack of statistically significant effects at higher dose levels, this observation is not considered to be toxicant related or biologically relevant.
LOQ = 0.25 µg ai/L

B. EFFECT ON REPRODUCTION

The mean time to first brood was 18.25, 18.5, 19, 18.5, and 18.75 days in the time-weighted average concentrations of 3.1, 6.8, 12, 27, and 49 µg ai/L, respectively, compared to 16.75 days in the negative control.

Mean offspring per surviving female was 13.9, 13.2, 14.2, 10.5, and 12.1 in the time-weighted average concentrations of 3.1, 6.8, 12, 27, and 49 µg ai/L groups, respectively, compared to 14.0 in the negative control. Based on reproduction, the NOAEC was 49 µg ai/L and the LOAEC was >49 µg ai/L.

At 96 hours, F_1 survival averaged 93, 90, 95, 98, and 95% in the time-weighted average concentrations of 3.1, 6.8, 12, 27, and 49 µg ai/L groups, respectively, compared to 95% in the negative control and 100% in the solvent control. Based on F_1 survival, the NOAEC was 2.9 µg ai/L and the LOAEC was >2.9 µg ai/L.

Table 4. Effect of Mesotrione on Reproduction of Adult and Survival of Young Mysid *sp.* [A]

Time-Weighted Average (Nominal) Concentration (µg ai/L)	Time to First Brood (Days) [B]	Reproduction (mean ± SD)		Percent Survival of F_1 Offspring (mean)
		Mean Offspring per Female	% of Females Producing Young	96 hours
Negative Control (<LOQ)	16.75	14.0±2.1	100	95
3.1 (3.1)	18.25	13.9±2.3	100	93
6.8 (6.3)	18.50	13.2±2.2	100	90
12 (13)	19.00	14.2±6.0	90	95
27 (25)	18.50	10.5±3.5	90	98
49 (50)	18.75	12.1±4.2	95	95
NOAEC, µg ai/L	Not analyzed	49	49	49
LOAEC, µg ai/L	Not analyzed	>49	>49	>49

[A] Data were obtained from Tables 4 and 7 on pages 34 and 37, respectively, of the study report.
[B] Calculated by Reviewers from raw data in Appendix 4 on pages 66-89 of the study report (Excel worksheet in Appendix I of the DER).
LOQ = 0.25 µg ai/L

C. REPORTED STATISTICS

Statistical analyses were performed on 28-day survival, male and female survival (post-pairing), growth (average dry body weight and average total body length) of both male and female mysids, and reproduction (number of young released per female).

The endpoint data were tested for normality using Shapiro-Wilk's test and for homogeneity using Bartlett's test. Data for the non-monotonic survival endpoints (28-day survival, male and female survival, and F1 survival) were analyzed using Fisher's Exact Test with Bonferroni-Holm's Adjustment. These endpoints met the assumptions of normal distribution and homogeneity. The one-sided Dunnett's Multiple Comparison test was used to evaluate non-monotonic data for length, dry weight, and total number of offspring per female. The NOAEC and LOAEC values were based on significance data, using scientific judgment to determine if statistical differences were biologically meaningful. No concentration resulted in >50% mortality when compared to the control data, and the LC_{50} value for survival was empirically estimated to be greater than the highest time-weighted average concentration. Analyses were performed using CETIS (Version 1.8; 2013), and results were reported based on time weighted average concentrations.

Parental Survival (Day 28)
LC_{50}: >49 µg ai/L 95% CI: N/A
NOAEC: 49 µg ai/L
LOAEC: >49 µg ai/L

Reproduction (offspring/female)
NOAEC: 49 µg ai/L
LOAEC: >49 µg ai/L

F_0 Length-Male (28 day)
NOAEC: 49 µg ai/L
LOAEC: >49 µg ai/L

F_0 Length-Female (28 day)
NOAEC: 49 µg ai/L
LOAEC: >49 µg ai/L

F_0 Dry Weight-Male (28 day)
NOAEC: 49 µg ai/L
LOAEC: >49 µg ai/L

F_0 Dry Weight - Female (28 day)
NOAEC: 49 µg ai/L
LOAEC: >49 µg ai/L

F_1 Survival
NOAEC: 49 µg ai/L
LOAEC: >49 µg ai/L

Endpoints Affected: None

D. VERIFICATION OF STATISTICAL RESULTS

The reviewer analyzed F_0 male and female growth (dry weight and length), F_0 mortality pre-pairing, F_0 mortality post-pairing, F_1 survival, time to first brood, and number of offspring per female data using CETIS statistical software version 1.9.2.8 with database backend settings implemented by EFED on 7/25/2017. Data were tested for normality using the Shapiro-Wilk's test ($\alpha = 0.01$) and for homogeneity of variance using Bartlett's or Levene's test ($\alpha = 0.01$). F_0 mortality post pairing and F_1 survival data did not meet assumptions and were analyzed using a nonparametric Mann-Whitney U Two-Sample test ($p<0.05$). All other endpoint data were analyzed using parametric Dunnett's or Williams Multiple Comparison test ($p<0.05$). Mean-measured concentrations calculated by the reviewer were used for analysis and reporting.

F_0 Female Dry Weight
NOAEC ≥ 50 µg ai/L
LOAEC >50 µg ai/L

F_0 Female Length*
NOAEC = 3.1 µg ai/L
LOAEC = 6.8 µg ai/L

F_0 Male Dry Weight
NOAEC ≥ 50 µg ai/L
LOAEC >50 µg ai/L

F_0 Male Length*
NOAEC = 26 µg ai/L
LOAEC = 50 µg ai/L

F_0 Mortality Post-Pairing
NOAEC ≥ 50 µg ai/L
LOAEC >50 µg ai/L

F_0 Mortality Pre-Pairing
NOAEC ≥ 50 µg ai/L
LOAEC >50 µg ai/L

F_1 Survival
NOAEC ≥ 50 µg ai/L
LOAEC >50 µg ai/L

Time to First Brood
NOAEC ≥ 50 µg ai/L
LOAEC >50 µg ai/L

Number of Offspring/Female**
NOAEC ≥ 50 µg ai/L
LOAEC > 50 µg ai/L

* Judged not likely treatment related (*see* Reviewer's Comments)
** NOAEC = 12 µg ai/L and LOAEC = 26 µg ai/L based on assumed biological significance (*see* Reviewer's Comments)

E. STUDY DEFICIENCIES

See above

F. REVIEWER'S COMMENTS

The reviewer identified statistically significant reductions for F0 female and male length in treatment groups compared to the negative control. In both cases, those reductions were slight (≤ 3%) relative to the negative control. The overall response appeared to be more dose-dependent in females than males but was relatively flat in both cases. Given the slight reduction and relatively flat dose-response it is not clear if the reduced length was treatment related or biologically relevant. EFED is not aware of any evidence to suggest a lack of biological relevance in regard to the observed magnitude of reduced length; therefore, the reviewer also took into consideration the results from the range finder to assess the likelihood that the reductions were treatment related. The range finder was conducted under conditions similar to the definitive test according to the study author and the range of test concentrations overlapped with the definitive test, making a direct comparison possible. It appears that the major difference between the range finder and definitive test was that the range finder used two replicates and the definitive test used four. In the range finder, 100% mortality was observed at nominal concentrations above 310 µg ai/L; therefore, length measurements were available for test concentrations of 10, 31, and 100 µg ai/L compared to length measurements in the definitive test at concentrations of 3.1 to 50 µg ai/L. This indicates that any trend in effects on length should be captured by the range of tested concentrations in the range finder and definitive tests. In the range finder (10 to 100 µg ai/L), both females and males showed increasing length with increasing concentration of mesotrione in contrast to the overall decreasing length of both sexes with increasing concentrations of mesotrione (3.3 to 50 µg ai/L) in the definitive test. In the case of range-finder females, the reduction ranged from 2% (10 µg ai/L) to 0% (100 µg ai/L) compared to the control. In the case of range-finder males, length was greater in the treatment groups compared to the control and increased with increasing concentration of mesotrione (0% to +4% from 10 to 100 µg ai/L). The reductions were about 2% to 3% for both sexes in the definitive test. Overall, it appears that the findings from the definitive test are not likely treatment related but rather background variability given the similar, low level of differences between the negative control and treatment groups in both the range finder and definitive test along with the lack of consistency in the directionality of the difference (*i.e.*, generally increasing reduction in length with increasing test concentration in the definitive test and the opposite in the range finder).

The reviewer, like the study author, did not detect a statistically significant difference among any of the treatment levels and the control for the number of offspring per female. However, the reviewer considers the NOAEC = 12 µg ai/L and the LOAEC = 26 µg ai/L based on potential biological significance of the magnitude of the reductions in the number of offspring (14% and 25%) at the two highest test concentrations in the definitive test. There is a clear shift in the level of reduction compared to the control between the 3.1 to 12 µg ai/L treatment levels (5% to + 1%) and the two higher concentrations that was not detected as significant by statistical analysis because the minimum significant difference that could be detected was 35% (William's test) to 45% (Dunnett's test) due to relatively high variability in the control and treatment groups. The inverted response (25% reduction at 26 µg ai/L and 14% at 50 µg ai/L) by itself adds to uncertainty regarding the likelihood that the observed reductions were treatment related. However, the results of the range finder test (*see* more details in the preceding paragraph) support the assumption that the observed reductions in the definitive test were treatment related. The number of offspring was reduced in a clear dose-responsive trend during the range finder (17%, 31%, and 78% in the 10, 31, and 100 µg ai/L groups, respectively; note that there was complete mortality at higher concentrations) at concentrations that overlapped and exceeded those showing reductions in the definitive test. Although there was a 17% reduction at 10 µg ai/L in the range finder compared to a +1% increase at 12 µg ai/L in the definitive test, the reviewer based the NOAEC (12 µg ai/L) only on the results of the definitive test given it had greater replication (4 vs 2 per treatment level). The LOAEC (26 µg ai/L) showed a similar reduction in the definitive test (25%) as the closest tested concentration in the range finder (31% reduction at 31 µg ai/L).

The reviewer's results are based on the mean-measured concentrations, whereas the study authors used time-weighted average concentrations even though analytical recoveries were stable throughout the test. A system malfunction occurred on Day 12 which left the exposure in a static state overnight, thus an additional Day 13 sampling interval was conducted. The Day 13 recoveries were 87 to 94% of nominal concentrations and demonstrated that mesotrione was stable under static test conditions. The scheduled Day 14 weekly sampling interval had recoveries ranging from 88 to 110% of nominal concentrations. The results from these two analytical intervals demonstrated that no interruption in exposure concentration occurred during the study. The reviewer's findings are presented in the Executive Summary and Conclusions sections of this DER.

The definitive testing dates were from April 27, 2016 to May 25, 2016.

G. CONCLUSIONS

This study is scientifically sound and is classified as **Acceptable**.

NOAEC = 12 µg ai/L
LOAEC = 26 µg ai/L based on reduced number of offspring per female

III. REFERENCES

None; other than standard guidelines and methodologies.

OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**			
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water		
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**			
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient	**Age:**	<24
Sample ID:	15-4661-0748	**Code:**	50096301	**Project:**	Herbicide		
Sample Date:	27 Apr-16	**Material:**	Mesotrione	**Source:**	Syngenta		
Receipt Date:	25 May-16	**CAS (PC):**		**Station:**			
Sample Age:	n/a	**Client:**	CDM Smith				

122990 50096301, mean-measured concentrations (ug ai/L), flow-through conditions, stats performed by A. Graff

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	TU	PMSD	S
17-4952-3817	F0 Female Dry Weight	Dunnett Multiple Comparison Test		50	>50	n/a		15.0%	1
05-9445-8116	F0 Female Dry Weight	Williams Multiple Comparison Test		50	>50	n/a		11.6%	1
16-2923-6272	F0 Female Length	Dunnett Multiple Comparison Test		50	>50	n/a		3.32%	1
11-5589-5117	F0 Female Length	Williams Multiple Comparison Test	✓	3.1	6.8	4.591		2.57%	1
02-5934-3733	F0 Male Dry Weight	Dunnett Multiple Comparison Test		50	>50	n/a		13.4%	1
05-2906-2503	F0 Male Dry Weight	Williams Multiple Comparison Test		50	>50	n/a		10.4%	1
07-8597-2934	F0 Male Length	Dunnett Multiple Comparison Test		6.8	12	9.033		3.11%	1
10-0023-2790	F0 Male Length	Williams Multiple Comparison Test		26	50	36.06		2.41%	1
17-4225-7050	F0 Mortality Post Pairing	Jonckheere-Terpstra Step-Down Test		50	>50	n/a		n/a	1
20-6839-2408	F0 Mortality Post Pairing	Mann-Whitney U Two-Sample Test		50	>50	n/a		13.5%	1
08-0446-2309	F0 Mortality Pre Pairing	Dunnett Multiple Comparison Test		50	>50	n/a		14.2%	1
01-1284-7247	F0 Mortality Pre Pairing	Williams Multiple Comparison Test		50	>50	n/a		11.0%	1
09-9968-8589	F1 Survival	Jonckheere-Terpstra Step-Down Test		50	>50	n/a		n/a	1
01-8201-7137	F1 Survival	Mann-Whitney U Two-Sample Test		50	>50	n/a		13.3%	1
17-5040-8922	n Offpspring Per Female	Dunnett Multiple Comparison Test		50	>50	n/a		44.7%	1
10-7456-4926	n Offpspring Per Female	Williams Multiple Comparison Test		50	>50	n/a		34.7%	1
21-1636-8021	Time to First Brood	Dunnett Multiple Comparison Test		50	>50	n/a		9.15%	1
07-8833-4348	Time to First Brood	Williams Multiple Comparison Test		50	>50	n/a		7.1%	1

OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

F0 Female Dry Weight Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	1.18	0.927	1.44	0.95	1.31	0.081	0.162	13.67%	0.00%
3.1		4	1.19	1.04	1.33	1.1	1.31	0.0457	0.0914	7.70%	-0.21%
6.8		4	1.24	1.12	1.35	1.15	1.32	0.0362	0.0723	5.86%	-4.22%
12		4	1.16	0.928	1.38	1.03	1.36	0.0712	0.142	12.34%	2.53%
26		4	1.19	1.12	1.26	1.13	1.24	0.0235	0.0469	3.94%	-0.42%
50		4	1.1	1.02	1.19	1.03	1.16	0.0272	0.0544	4.93%	6.96%

F0 Female Length Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	7.84	7.65	8.04	7.75	8.02	0.0606	0.121	1.55%	0.00%
3.1		4	7.73	7.45	8.01	7.57	7.97	0.0882	0.176	2.28%	1.50%
6.8		4	7.64	7.44	7.83	7.53	7.81	0.0608	0.122	1.59%	2.68%
12		4	7.65	7.58	7.71	7.6	7.7	0.0206	0.0411	0.54%	2.52%
26		4	7.64	7.29	7.98	7.42	7.93	0.108	0.217	2.84%	2.64%
50		4	7.6	7.32	7.89	7.44	7.79	0.088	0.176	2.31%	3.06%

F0 Male Dry Weight Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	0.88	0.742	1.02	0.75	0.93	0.0434	0.0868	9.86%	0.00%
3.1		4	0.86	0.792	0.928	0.81	0.9	0.0212	0.0424	4.93%	2.27%
6.8		4	0.87	0.82	0.92	0.84	0.91	0.0158	0.0316	3.63%	1.14%
12		4	0.895	0.749	1.04	0.83	1.03	0.0457	0.0915	10.22%	-1.70%
26		4	0.822	0.71	0.935	0.77	0.92	0.0354	0.0709	8.62%	6.53%
50		4	0.9	0.787	1.01	0.8	0.96	0.0356	0.0712	7.91%	-2.27%

F0 Male Length Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	7.68	7.47	7.89	7.5	7.79	0.0665	0.133	1.73%	0.00%
3.1		4	7.54	7.44	7.64	7.45	7.58	0.0308	0.0616	0.82%	1.82%
6.8		4	7.54	7.4	7.69	7.42	7.62	0.0452	0.0903	1.20%	1.79%
12		4	7.42	7.33	7.51	7.37	7.48	0.0281	0.0562	0.76%	3.42%
26		4	7.58	7.16	8	7.25	7.87	0.133	0.267	3.52%	1.30%
50		4	7.44	7.25	7.63	7.3	7.59	0.0594	0.119	1.60%	3.09%

F0 Mortality Post Pairing Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	0.145	0.098	0.193	0.118	0.188	0.015	0.030	20.62%	0.00%
3.1		4	0.107	0.038	0.176	0.053	0.158	0.022	0.044	40.75%	-4.48%
6.8		4	0.159	0.072	0.246	0.105	0.235	0.027	0.055	34.37%	1.64%
12		4	0.080	0.047	0.113	0.067	0.111	0.010	0.021	25.89%	-7.63%
26		4	0.263	0.000	0.540	0.067	0.429	0.087	0.174	66.33%	13.73%
50		4	0.159	0.038	0.280	0.067	0.250	0.038	0.076	47.86%	1.60%

F0 Mortality Pre Pairing Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	0.114	0.075	0.152	0.100	0.150	0.012	0.024	21.31%	0.00%
3.1		4	0.063	0.000	0.163	0.000	0.150	0.032	0.063	100.66%	-5.79%
6.8		4	0.112	0.000	0.289	0.000	0.250	0.055	0.111	98.55%	-0.15%
12		4	0.100	0.000	0.245	0.000	0.200	0.046	0.091	91.29%	-1.56%
26		4	0.075	0.000	0.178	0.000	0.150	0.032	0.065	86.07%	-4.38%
50		4	0.100	0.008	0.192	0.050	0.150	0.029	0.058	57.74%	-1.56%

OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

F1 Survival Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	0.950	0.858	1.000	0.900	1.000	0.029	0.058	6.08%	0.00%
3.1		3	0.933	0.646	1.000	0.800	1.000	0.067	0.115	12.37%	1.75%
6.8		4	0.900	0.716	1.000	0.800	1.000	0.058	0.115	12.83%	5.26%
12		4	0.950	0.791	1.000	0.800	1.000	0.050	0.100	10.53%	0.00%
26		4	0.975	0.895	1.000	0.900	1.000	0.025	0.050	5.13%	-2.63%
50		4	0.950	0.791	1.000	0.800	1.000	0.050	0.100	10.53%	0.00%

n Offpspring Per Female Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	14	10.6	17.4	11.4	16.2	1.07	2.14	15.25%	0.00%
3.1		4	13.9	10.3	17.5	10.6	15.6	1.13	2.25	16.22%	0.71%
6.8		4	13.2	9.7	16.7	10	15	1.1	2.2	16.64%	5.71%
12		4	14.2	4.58	23.8	7.2	21.4	3.02	6.05	42.58%	-1.43%
26		4	10.5	4.9	16.1	6	13.4	1.76	3.52	33.52%	25.00%
50		4	12	5.37	18.7	6.2	15.8	2.1	4.2	34.85%	13.93%

Time to First Brood Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	4	19.4	17.4	21.3	18	20.8	0.608	1.22	6.28%	0.00%
3.1		4	20.3	19.2	21.5	19.4	21.2	0.369	0.737	3.62%	-5.17%
6.8		4	20.3	18.2	22.4	18.8	21.8	0.645	1.29	6.36%	-4.91%
12		4	20.3	19	21.6	19.6	21.5	0.411	0.822	4.04%	-5.04%
26		4	20.7	19.7	21.7	20.2	21.6	0.325	0.649	3.14%	-6.98%
50		4	20.6	18.5	22.6	18.8	21.8	0.654	1.31	6.36%	-6.27%

OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

F0 Female Dry Weight Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	1.21	1.27	0.95	1.31
3.1		1.14	1.31	1.1	1.2
6.8		1.15	1.26	1.21	1.32
12		1.03	1.12	1.11	1.36
26		1.21	1.13	1.18	1.24
50		1.16	1.12	1.1	1.03

F0 Female Length Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	7.78	7.83	7.75	8.02
3.1		7.63	7.97	7.74	7.57
6.8		7.59	7.61	7.81	7.53
12		7.64	7.7	7.65	7.6
26		7.65	7.93	7.42	7.55
50		7.47	7.79	7.72	7.44

F0 Male Dry Weight Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	0.75	0.93	0.92	0.92
3.1		0.84	0.9	0.81	0.89
6.8		0.88	0.85	0.84	0.91
12		1.03	0.85	0.83	0.87
26		0.83	0.77	0.77	0.92
50		0.8	0.94	0.9	0.96

F0 Male Length Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	7.5	7.77	7.79	7.66
3.1		7.45	7.58	7.58	7.55
6.8		7.42	7.53	7.62	7.6
12		7.37	7.37	7.48	7.45
26		7.5	7.7	7.87	7.25
50		7.3	7.59	7.43	7.45

F0 Mortality Post Pairing Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	0.118	0.188	0.133	0.143
3.1		0.100	0.158	0.118	0.053
6.8		0.143	0.235	0.105	0.154
12		0.071	0.111	0.067	0.071
26		0.067	0.389	0.429	0.167
50		0.067	0.250	0.143	0.176

F0 Mortality Pre Pairing Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	0.105	0.100	0.150	0.100
3.1		0.000	0.050	0.150	0.050
6.8		0.250	0.050	0.000	0.150
12		0.200	0.050	0.000	0.150
26		0.100	0.000	0.150	0.050
50		0.050	0.150	0.150	0.050

OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

F1 Survival Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	0.900	0.900	1.000	1.000
3.1		1.000	0.800	1.000	
6.8		0.800	1.000	1.000	0.800
12		0.800	1.000	1.000	1.000
26		0.900	1.000	1.000	1.000
50		1.000	1.000	1.000	0.800

n Offpspring Per Female Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	15.2	13.2	16.2	11.4
3.1		15.6	15	14.4	10.6
6.8		15	13.8	10	14
12		21.4	16.2	7.2	12
26		13.2	6	13.4	9.4
50		14.2	15.8	12	6.2

Time to First Brood Detail

Conc-µg ai/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	N	18.8	19.8	18	20.8
3.1		19.4	20.4	20.4	21.2
6.8		21.8	19.8	20.8	18.8
12		19.6	20	21.5	20.2
26		20.2	20.8	21.6	20.2
50		21.8	18.8	20.4	21.2

CETIS Analytical Report

OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

Analysis ID:	17-4952-3817	**Endpoint:**	F0 Female Dry Weight	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	50	>50	n/a		14.98%

Dunnett Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	-0.0339	2.41	0.178	6	CDF	0.8431	Non-Significant Effect
	6.8	-0.678	2.41	0.178	6	CDF	0.9604	Non-Significant Effect
	12	0.407	2.41	0.178	6	CDF	0.6872	Non-Significant Effect
	26	-0.0678	2.41	0.178	6	CDF	0.8525	Non-Significant Effect
	50	1.12	2.41	0.178	6	CDF	0.3707	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0389333	0.0077867	5	0.716	0.6199	Non-Significant Effect
Error	0.19585	0.0108806	18			
Total	0.234783		23			

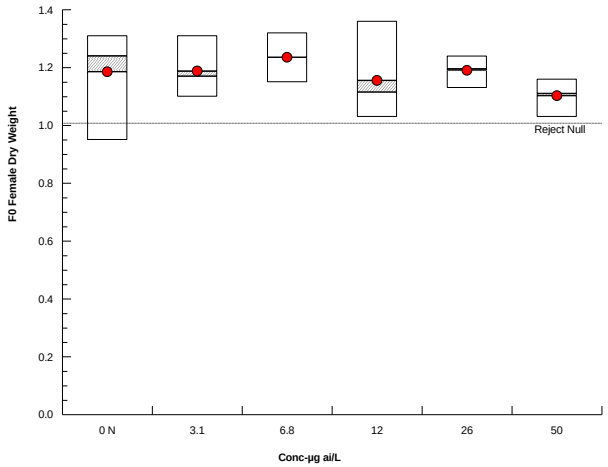
ANOVA Assumptions Tests

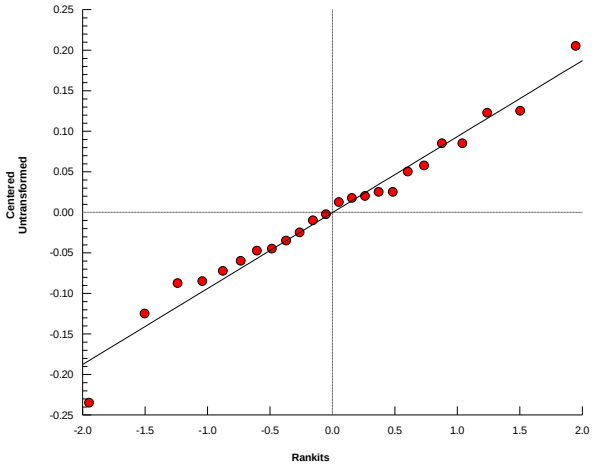
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.35	15.1	0.2732	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.982	0.884	0.9278	Normal Distribution

F0 Female Dry Weight Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	1.18	0.927	1.44	1.24	0.95	1.31	0.081	13.67%	0.00%
3.1		4	1.19	1.04	1.33	1.17	1.1	1.31	0.0457	7.70%	-0.21%
6.8		4	1.24	1.12	1.35	1.24	1.15	1.32	0.0362	5.86%	-4.22%
12		4	1.16	0.928	1.38	1.12	1.03	1.36	0.0712	12.34%	2.53%
26		4	1.19	1.12	1.26	1.19	1.13	1.24	0.0235	3.94%	-0.42%
50		4	1.1	1.02	1.19	1.11	1.03	1.16	0.0272	4.93%	6.96%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

Analysis ID:	05-9445-8116	**Endpoint:**	F0 Female Dry Weight	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	50	>50	n/a		11.62%

Williams Multiple Comparison Test

Control	vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		3.1	-0.0339	1.73	0.128	6	CDF	>0.05	Non-Significant Effect
		6.8	-0.356	1.82	0.134	6	CDF	>0.05	Non-Significant Effect
		12	0.407	1.85	0.136	6	CDF	>0.05	Non-Significant Effect
		26	0.169	1.86	0.137	6	CDF	>0.05	Non-Significant Effect
		50	1.12	1.87	0.138	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0389333	0.0077867	5	0.716	0.6199	Non-Significant Effect
Error	0.19585	0.0108806	18			
Total	0.234783		23			

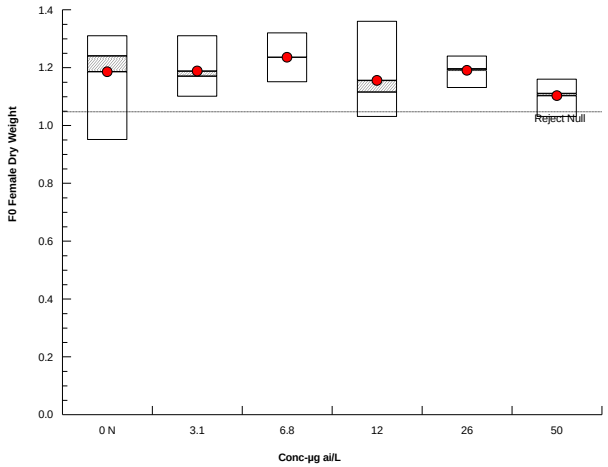
ANOVA Assumptions Tests

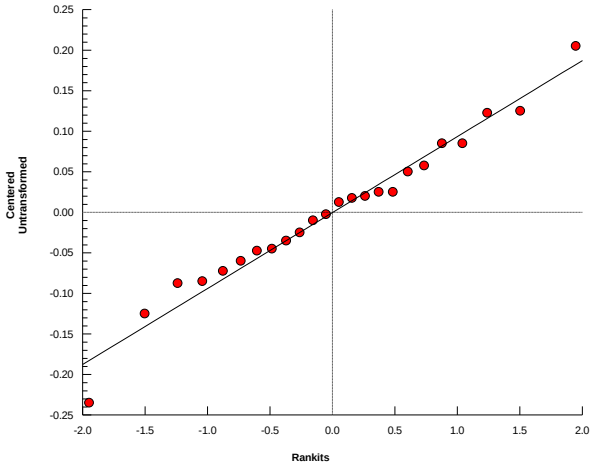
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.35	15.1	0.2732	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.982	0.884	0.9278	Normal Distribution

F0 Female Dry Weight Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	1.18	0.927	1.44	1.24	0.95	1.31	0.081	13.67%	0.00%
3.1		4	1.19	1.04	1.33	1.17	1.1	1.31	0.0457	7.70%	-0.21%
6.8		4	1.24	1.12	1.35	1.24	1.15	1.32	0.0362	5.86%	-4.22%
12		4	1.16	0.928	1.38	1.12	1.03	1.36	0.0712	12.34%	2.53%
26		4	1.19	1.12	1.26	1.19	1.13	1.24	0.0235	3.94%	-0.42%
50		4	1.1	1.02	1.19	1.11	1.03	1.16	0.0272	4.93%	6.96%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) — **Smithers Viscient**

Analysis ID:	16-2923-6272	**Endpoint:**	F0 Female Length	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	50	>50	n/a		3.32%

Dunnett Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	1.09	2.41	0.26	6	CDF	0.3839	Non-Significant Effect
	6.8	1.94	2.41	0.26	6	CDF	0.1147	Non-Significant Effect
	12	1.83	2.41	0.26	6	CDF	0.1388	Non-Significant Effect
	26	1.92	2.41	0.26	6	CDF	0.1192	Non-Significant Effect
	50	2.22	2.41	0.26	6	CDF	0.0706	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.159771	0.0319542	5	1.37	0.2822	Non-Significant Effect
Error	0.420325	0.0233514	18			
Total	0.580096		23			

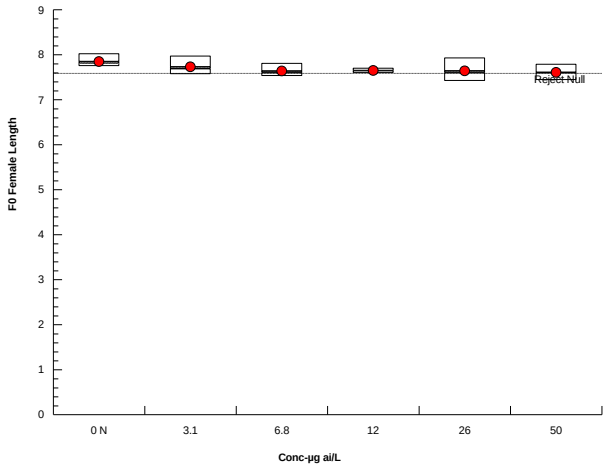
ANOVA Assumptions Tests

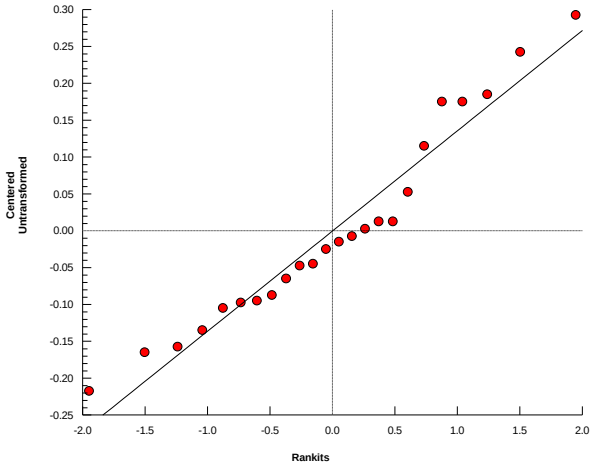
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.06	15.1	0.3008	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.946	0.884	0.2194	Normal Distribution

F0 Female Length Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	7.85	7.65	8.04	7.81	7.75	8.02	0.0606	1.55%	0.00%
3.1		4	7.73	7.45	8.01	7.68	7.57	7.97	0.0882	2.28%	1.50%
6.8		4	7.64	7.44	7.83	7.6	7.53	7.81	0.0608	1.59%	2.68%
12		4	7.65	7.58	7.71	7.64	7.6	7.7	0.0206	0.54%	2.52%
26		4	7.64	7.29	7.98	7.6	7.42	7.93	0.108	2.84%	2.65%
50		4	7.6	7.32	7.89	7.59	7.44	7.79	0.088	2.31%	3.06%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

Analysis ID:	11-5589-5117	**Endpoint:**	F0 Female Length	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	3.1	6.8	4.591		2.57%

Williams Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	1.09	1.73	0.187	6	CDF	>0.05	Non-Significant Effect
	6.8*	1.94	1.82	0.196	6	CDF	<0.05	Significant Effect
	12*	1.89	1.85	0.199	6	CDF	<0.05	Significant Effect
	26*	1.92	1.86	0.201	6	CDF	<0.05	Significant Effect
	50*	2.22	1.87	0.202	6	CDF	<0.05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.159771	0.0319542	5	1.37	0.2822	Non-Significant Effect
Error	0.420325	0.0233514	18			
Total	0.580096		23			

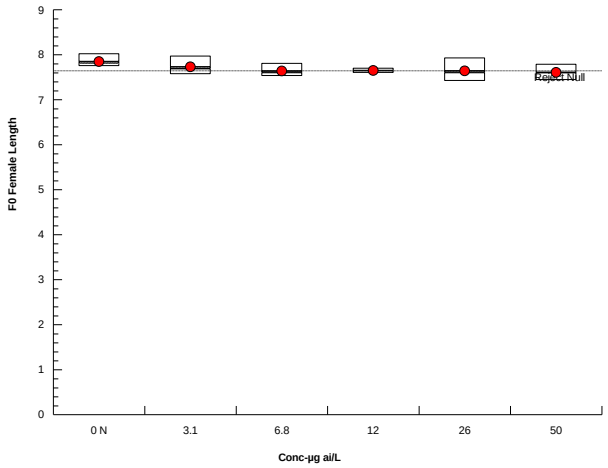
ANOVA Assumptions Tests

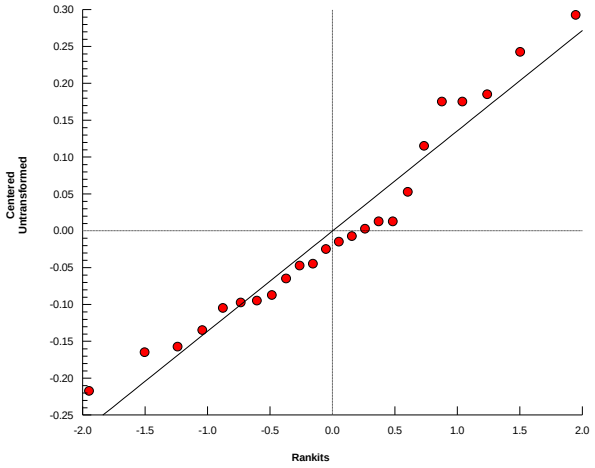
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.06	15.1	0.3008	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.946	0.884	0.2194	Normal Distribution

F0 Female Length Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	7.85	7.65	8.04	7.81	7.75	8.02	0.0606	1.55%	0.00%
3.1		4	7.73	7.45	8.01	7.68	7.57	7.97	0.0882	2.28%	1.50%
6.8		4	7.64	7.44	7.83	7.6	7.53	7.81	0.0608	1.59%	2.68%
12		4	7.65	7.58	7.71	7.64	7.6	7.7	0.0206	0.54%	2.52%
26		4	7.64	7.29	7.98	7.6	7.42	7.93	0.108	2.84%	2.65%
50		4	7.6	7.32	7.89	7.59	7.44	7.79	0.088	2.31%	3.06%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) — **Smithers Viscient**

Analysis ID:	02-5934-3733	**Endpoint:**	F0 Male Dry Weight	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	50	>50	n/a		13.40%

Dunnett Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	0.408	2.41	0.118	6	CDF	0.6866	Non-Significant Effect
	6.8	0.204	2.41	0.118	6	CDF	0.7662	Non-Significant Effect
	12	-0.306	2.41	0.118	6	CDF	0.9077	Non-Significant Effect
	26	1.17	2.41	0.118	6	CDF	0.3477	Non-Significant Effect
	50	-0.408	2.41	0.118	6	CDF	0.9259	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0158875	0.0031775	5	0.662	0.6567	Non-Significant Effect
Error	0.086375	0.0047986	18			
Total	0.102262		23			

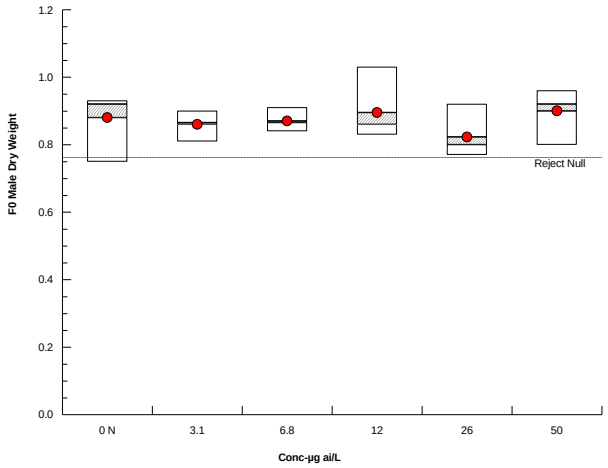
ANOVA Assumptions Tests

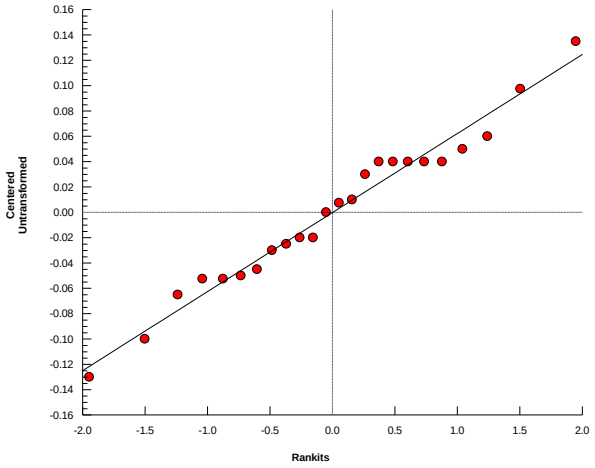
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	3.83	15.1	0.5744	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.979	0.884	0.8815	Normal Distribution

F0 Male Dry Weight Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.88	0.742	1.02	0.92	0.75	0.93	0.0434	9.86%	0.00%
3.1		4	0.86	0.792	0.928	0.865	0.81	0.9	0.0212	4.93%	2.27%
6.8		4	0.87	0.82	0.92	0.865	0.84	0.91	0.0158	3.63%	1.14%
12		4	0.895	0.749	1.04	0.86	0.83	1.03	0.0457	10.22%	-1.70%
26		4	0.822	0.71	0.935	0.8	0.77	0.92	0.0354	8.62%	6.53%
50		4	0.9	0.787	1.01	0.92	0.8	0.96	0.0356	7.91%	-2.27%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) — **Smithers Viscient**

Analysis ID:	05-2906-2503	**Endpoint:**	F0 Male Dry Weight	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	50	>50	n/a		10.39%

Williams Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	0.408	1.73	0.085	6	CDF	>0.05	Non-Significant Effect
	6.8	0.306	1.82	0.089	6	CDF	>0.05	Non-Significant Effect
	12	0.102	1.85	0.090	6	CDF	>0.05	Non-Significant Effect
	26	1.17	1.86	0.091	6	CDF	>0.05	Non-Significant Effect
	50	0.383	1.87	0.092	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0158875	0.0031775	5	0.662	0.6567	Non-Significant Effect
Error	0.086375	0.0047986	18			
Total	0.102262		23			

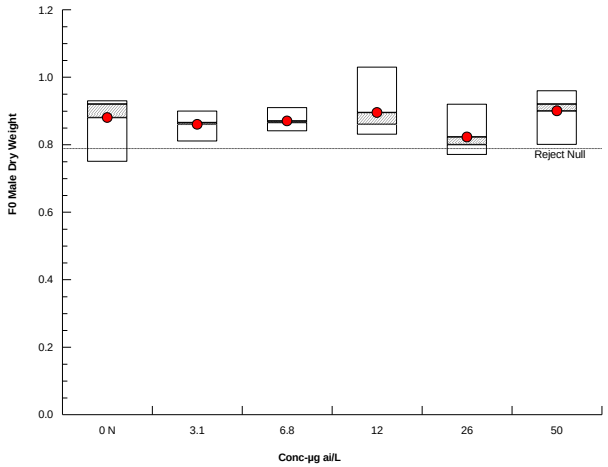
ANOVA Assumptions Tests

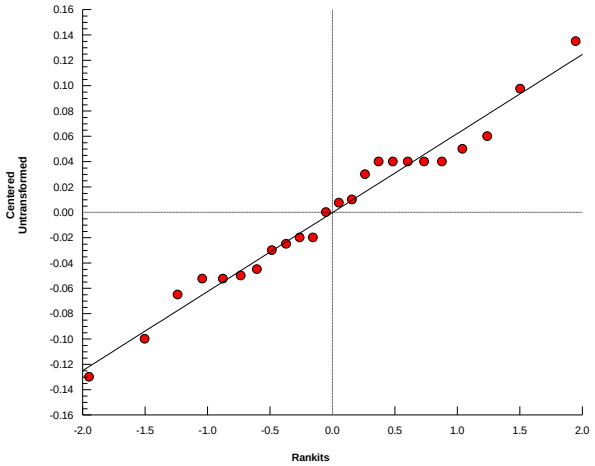
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	3.83	15.1	0.5744	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.979	0.884	0.8815	Normal Distribution

F0 Male Dry Weight Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.88	0.742	1.02	0.92	0.75	0.93	0.0434	9.86%	0.00%
3.1		4	0.86	0.792	0.928	0.865	0.81	0.9	0.0212	4.93%	2.27%
6.8		4	0.87	0.82	0.92	0.865	0.84	0.91	0.0158	3.63%	1.14%
12		4	0.895	0.749	1.04	0.86	0.83	1.03	0.0457	10.22%	-1.70%
26		4	0.822	0.71	0.935	0.8	0.77	0.92	0.0354	8.62%	6.53%
50		4	0.9	0.787	1.01	0.92	0.8	0.96	0.0356	7.91%	-2.27%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

Analysis ID:	07-8597-2934	**Endpoint:**	F0 Male Length	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	6.8	12	9.033		3.11%

Dunnett Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	1.41	2.41	0.239	6	CDF	0.2577	Non-Significant Effect
	6.8	1.39	2.41	0.239	6	CDF	0.2666	Non-Significant Effect
	12*	2.65	2.41	0.239	6	CDF	0.0315	Significant Effect
	26	1.01	2.41	0.239	6	CDF	0.4184	Non-Significant Effect
	50	2.39	2.41	0.239	6	CDF	0.0513	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.181938	0.0363875	5	1.85	0.1541	Non-Significant Effect
Error	0.354425	0.0196903	18			
Total	0.536363		23			

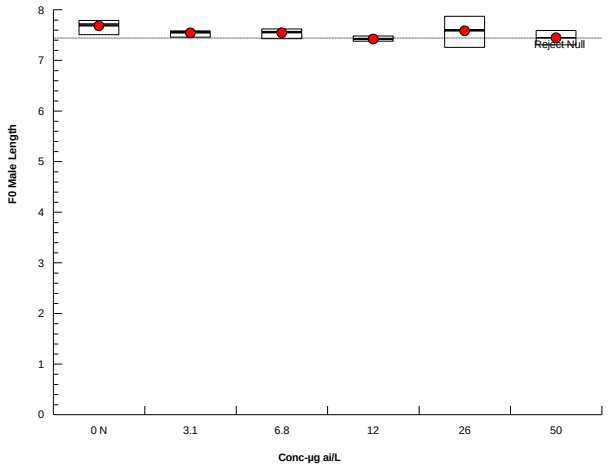
ANOVA Assumptions Tests

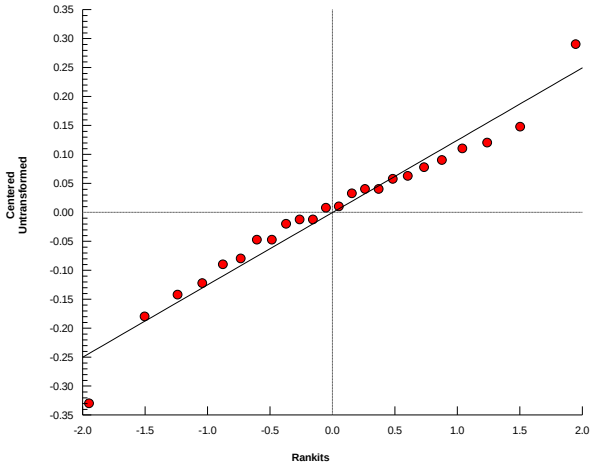
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	9.33	15.1	0.0966	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.969	0.884	0.6480	Normal Distribution

F0 Male Length Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	7.68	7.47	7.89	7.72	7.5	7.79	0.0665	1.73%	0.00%
3.1		4	7.54	7.44	7.64	7.57	7.45	7.58	0.0308	0.82%	1.82%
6.8		4	7.54	7.4	7.69	7.57	7.42	7.62	0.0452	1.20%	1.79%
12		4	7.42	7.33	7.51	7.41	7.37	7.48	0.0281	0.76%	3.42%
26		4	7.58	7.16	8	7.6	7.25	7.87	0.133	3.52%	1.30%
50		4	7.44	7.25	7.63	7.44	7.3	7.59	0.0594	1.59%	3.09%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) — **Smithers Viscient**

Analysis ID:	10-0023-2790	**Endpoint:**	F0 Male Length	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	26	50	36.06		2.41%

Williams Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	1.41	1.73	0.172	6	CDF	>0.05	Non-Significant Effect
	6.8	1.4	1.82	0.18	6	CDF	>0.05	Non-Significant Effect
	12	2.65	1.85	0.183	6	CDF	>0.05	Non-Significant Effect
	26	1.83	1.86	0.184	6	CDF	>0.05	Non-Significant Effect
	50*	2.39	1.87	0.185	6	CDF	<0.05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.181938	0.0363875	5	1.85	0.1541	Non-Significant Effect
Error	0.354425	0.0196903	18			
Total	0.536363		23			

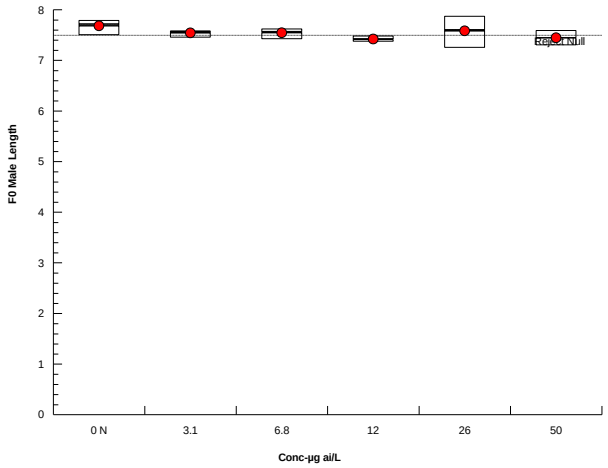
ANOVA Assumptions Tests

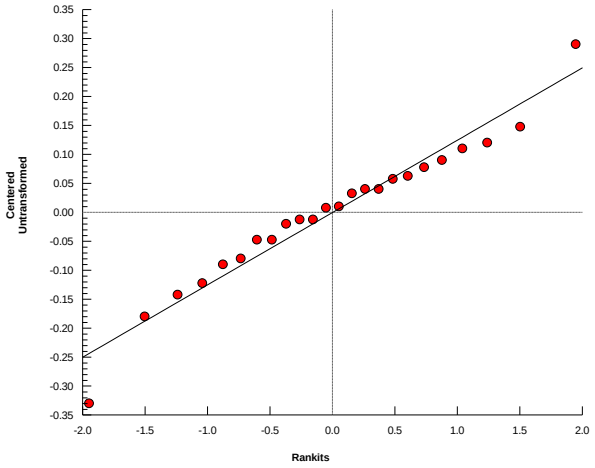
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	9.33	15.1	0.0966	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.969	0.884	0.6480	Normal Distribution

F0 Male Length Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	7.68	7.47	7.89	7.72	7.5	7.79	0.0665	1.73%	0.00%
3.1		4	7.54	7.44	7.64	7.57	7.45	7.58	0.0308	0.82%	1.82%
6.8		4	7.54	7.4	7.69	7.57	7.42	7.62	0.0452	1.20%	1.79%
12		4	7.42	7.33	7.51	7.41	7.37	7.48	0.0281	0.76%	3.42%
26		4	7.58	7.16	8	7.6	7.25	7.87	0.133	3.52%	1.30%
50		4	7.44	7.25	7.63	7.44	7.3	7.59	0.0594	1.59%	3.09%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

Analysis ID:	20-6839-2408	**Endpoint:**	F0 Mortality Post Pairing	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:55	**Analysis:**	Nonparametric-Two Sample	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C < T	50	>50	n/a		13.51%

Mann-Whitney U Two-Sample Test

Control	vs	Conc-µg ai/L	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		3.1	3.5	n/a	1	6	Exact	0.9143	Non-Significant Effect
		6.8	9.5	n/a	1	6	Exact	0.3714	Non-Significant Effect
		12	0	n/a	0	6	Exact	1.0000	Non-Significant Effect
		26	11	n/a	0	6	Exact	0.2429	Non-Significant Effect
		50	9.5	n/a	1	6	Exact	0.3714	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0783317	0.0156663	5	2.22	0.0973	Non-Significant Effect
Error	0.127152	0.007064	18			
Total	0.205483		23			

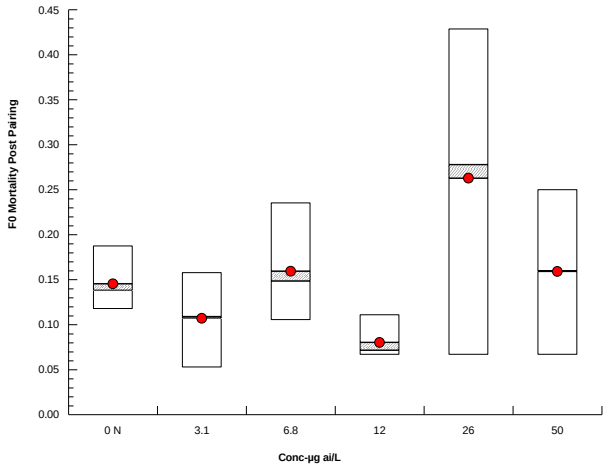
ANOVA Assumptions Tests

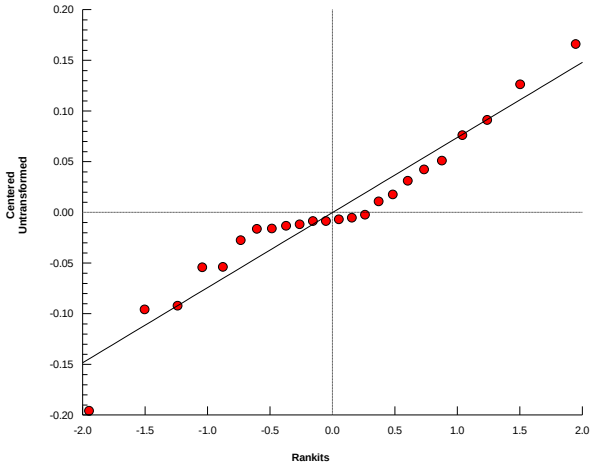
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	15.3	15.1	0.0091	Unequal Variances
Distribution	Shapiro-Wilk W Normality Test	0.949	0.884	0.2633	Normal Distribution

F0 Mortality Post Pairing Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.145	0.098	0.193	0.138	0.118	0.188	0.015	20.62%	0.00%
3.1		4	0.107	0.038	0.176	0.109	0.053	0.158	0.022	40.75%	-4.48%
6.8		4	0.159	0.072	0.246	0.148	0.105	0.235	0.027	34.37%	1.64%
12		4	0.080	0.047	0.113	0.071	0.067	0.111	0.010	25.89%	-7.63%
26		4	0.263	0.000	0.540	0.278	0.067	0.429	0.087	66.33%	13.73%
50		4	0.159	0.038	0.280	0.160	0.067	0.250	0.038	47.86%	1.60%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) — **Smithers Viscient**

Analysis ID:	17-4225-7050	**Endpoint:**	F0 Mortality Post Pairing	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:56	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C < T	50	>50	n/a	

Jonckheere-Terpstra Step-Down Test

Control vs	Conc-µg ai/L	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	-1.31	1.64	1	Asymp	0.9443	Non-Significant Effect
	6.8	0.147	1.64	2	Asymp	0.9443	Non-Significant Effect
	12	-1.59	1.64	3	Asymp	0.9443	Non-Significant Effect
	26	-0.0998	1.64	4	Asymp	0.5398	Non-Significant Effect
	50	0.329	1.64	4	Asymp	0.3712	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0783317	0.0156663	5	2.22	0.0973	Non-Significant Effect
Error	0.127152	0.007064	18			
Total	0.205483		23			

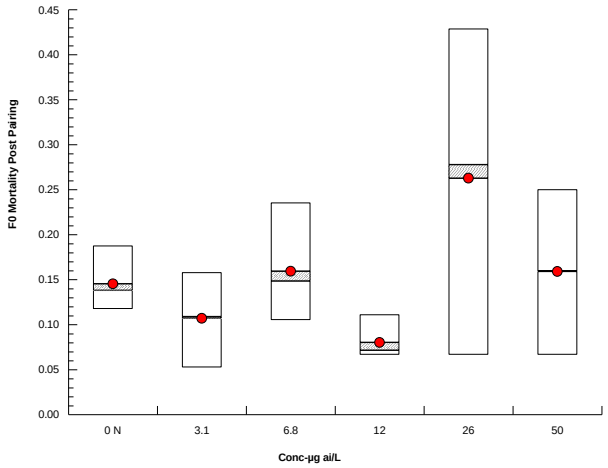
ANOVA Assumptions Tests

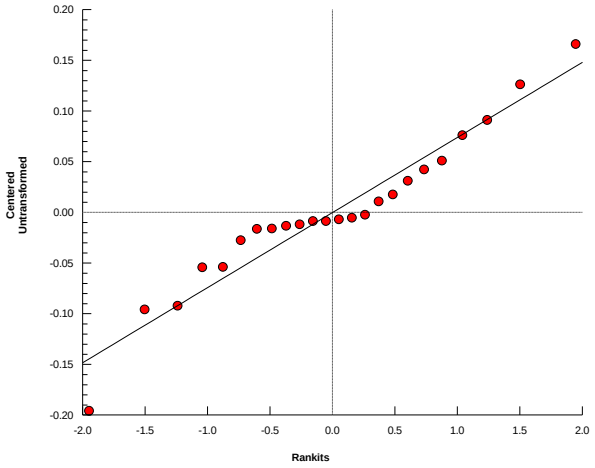
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	15.3	15.1	0.0091	Unequal Variances
Distribution	Shapiro-Wilk W Normality Test	0.949	0.884	0.2633	Normal Distribution

F0 Mortality Post Pairing Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.145	0.098	0.193	0.138	0.118	0.188	0.015	20.62%	0.00%
3.1		4	0.107	0.038	0.176	0.109	0.053	0.158	0.022	40.75%	-4.48%
6.8		4	0.159	0.072	0.246	0.148	0.105	0.235	0.027	34.37%	1.64%
12		4	0.080	0.047	0.113	0.071	0.067	0.111	0.010	25.89%	-7.63%
26		4	0.263	0.000	0.540	0.278	0.067	0.429	0.087	66.33%	13.73%
50		4	0.159	0.038	0.280	0.160	0.067	0.250	0.038	47.86%	1.60%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

Analysis ID:	08-0446-2309	**Endpoint:**	F0 Mortality Pre Pairing	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:55	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C < T	50	>50	n/a		14.17%

Dunnett Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	-0.983	2.41	0.126	6	CDF	0.9820	Non-Significant Effect
	6.8	-0.0252	2.41	0.126	6	CDF	0.8407	Non-Significant Effect
	12	-0.265	2.41	0.126	6	CDF	0.8995	Non-Significant Effect
	26	-0.744	2.41	0.126	6	CDF	0.9663	Non-Significant Effect
	50	-0.265	2.41	0.126	6	CDF	0.8995	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0086406	0.0017281	5	0.317	0.8960	Non-Significant Effect
Error	0.0980142	0.0054452	18			
Total	0.106655		23			

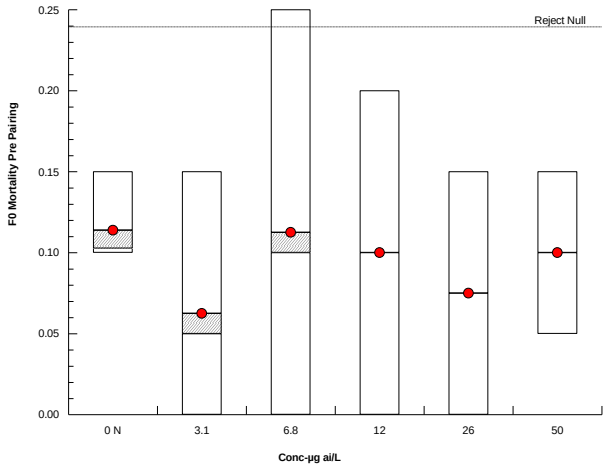
ANOVA Assumptions Tests

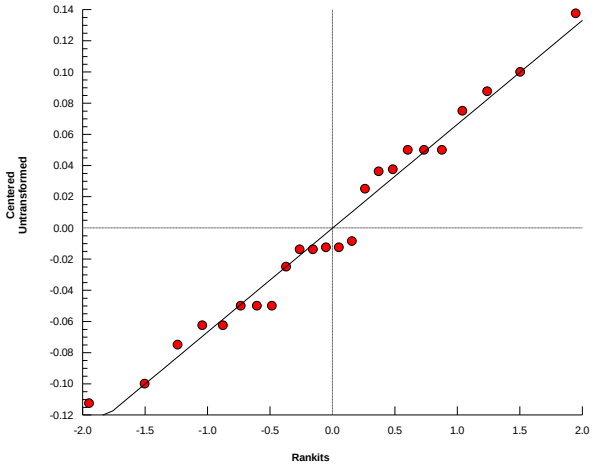
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	5.48	15.1	0.3602	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.974	0.884	0.7585	Normal Distribution

F0 Mortality Pre Pairing Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.114	0.075	0.152	0.103	0.100	0.150	0.012	21.31%	0.00%
3.1		4	0.063	0.000	0.163	0.050	0.000	0.150	0.032	100.66%	-5.79%
6.8		4	0.113	0.000	0.289	0.100	0.000	0.250	0.055	98.55%	-0.15%
12		4	0.100	0.000	0.245	0.100	0.000	0.200	0.046	91.29%	-1.56%
26		4	0.075	0.000	0.178	0.075	0.000	0.150	0.032	86.07%	-4.38%
50		4	0.100	0.008	0.192	0.100	0.050	0.150	0.029	57.74%	-1.56%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) — **Smithers Viscient**

Analysis ID:	01-1284-7247	**Endpoint:**	F0 Mortality Pre Pairing	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:56	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C < T	50	>50	n/a		10.99%

Williams Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	-0.983	1.73	0.091	6	CDF	>0.05	Non-Significant Effect
	6.8	-0.0252	1.82	0.095	6	CDF	>0.05	Non-Significant Effect
	12	-0.145	1.85	0.096	6	CDF	>0.05	Non-Significant Effect
	26	-0.345	1.86	0.097	6	CDF	>0.05	Non-Significant Effect
	50	-0.265	1.87	0.097	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0086406	0.0017281	5	0.317	0.8960	Non-Significant Effect
Error	0.0980142	0.0054452	18			
Total	0.106655		23			

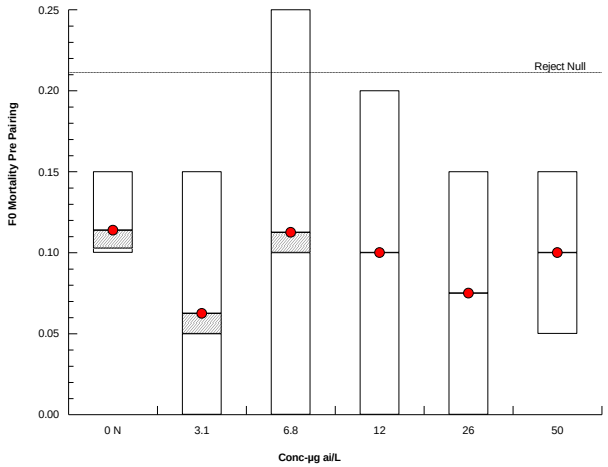
ANOVA Assumptions Tests

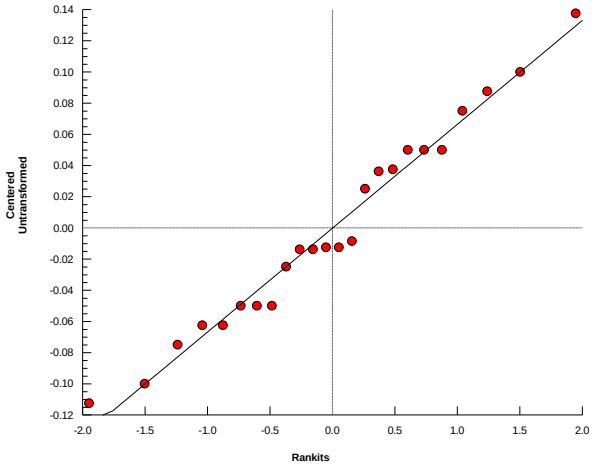
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	5.48	15.1	0.3602	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.974	0.884	0.7585	Normal Distribution

F0 Mortality Pre Pairing Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.114	0.075	0.152	0.103	0.100	0.150	0.012	21.31%	0.00%
3.1		4	0.063	0.000	0.163	0.050	0.000	0.150	0.032	100.66%	-5.79%
6.8		4	0.113	0.000	0.289	0.100	0.000	0.250	0.055	98.55%	-0.15%
12		4	0.100	0.000	0.245	0.100	0.000	0.200	0.046	91.29%	-1.56%
26		4	0.075	0.000	0.178	0.075	0.000	0.150	0.032	86.07%	-4.38%
50		4	0.100	0.008	0.192	0.100	0.050	0.150	0.029	57.74%	-1.56%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) — **Smithers Viscient**

Analysis ID:	01-8201-7137	**Endpoint:**	F1 Survival	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Nonparametric-Two Sample	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	50	>50	n/a		13.32%

Mann-Whitney U Two-Sample Test

Control	vs	Conc-µg ai/L	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		3.1	6	n/a	2	5	Exact	0.6286	Non-Significant Effect
		6.8	10	n/a	2	6	Exact	0.3286	Non-Significant Effect
		12	7	n/a	2	6	Exact	0.6429	Non-Significant Effect
		26	6	n/a	3	6	Exact	0.9286	Non-Significant Effect
		50	7	n/a	2	6	Exact	0.6429	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0123551	0.0024710	5	0.291	0.9112	Non-Significant Effect
Error	0.144167	0.0084804	17			
Total	0.156522		22			

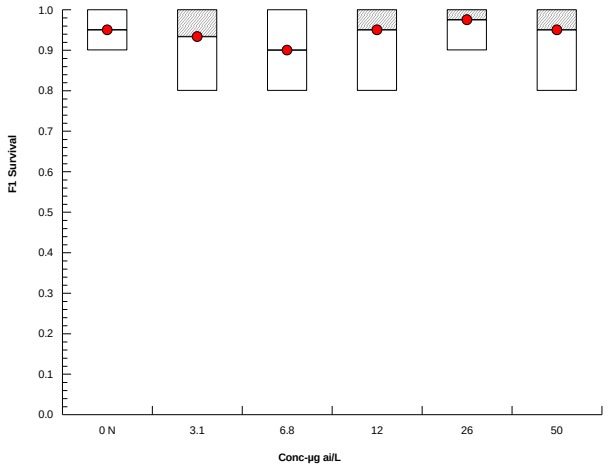
ANOVA Assumptions Tests

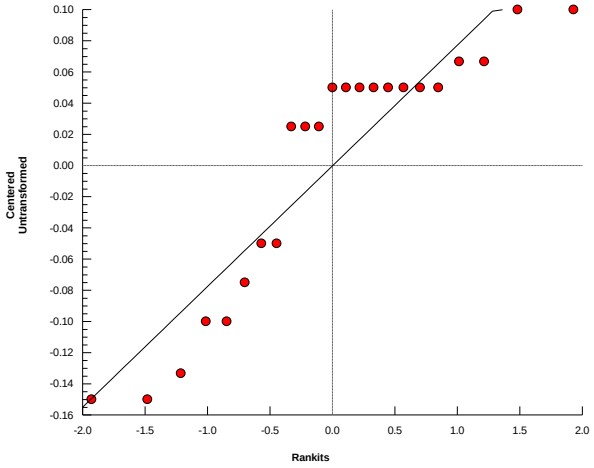
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	2.82	15.1	0.7280	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.844	0.88	0.0021	Non-Normal Distribution

F1 Survival Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.950	0.858	1.000	0.950	0.900	1.000	0.029	6.08%	0.00%
3.1		3	0.933	0.646	1.000	1.000	0.800	1.000	0.067	12.37%	1.75%
6.8		4	0.900	0.716	1.000	0.900	0.800	1.000	0.058	12.83%	5.26%
12		4	0.950	0.791	1.000	1.000	0.800	1.000	0.050	10.53%	0.00%
26		4	0.975	0.895	1.000	1.000	0.900	1.000	0.025	5.13%	-2.63%
50		4	0.950	0.791	1.000	1.000	0.800	1.000	0.050	10.53%	0.00%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

Analysis ID:	09-9968-8589	**Endpoint:**	F1 Survival	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Nonparametric-Control vs Ord. Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C > T	50	>50	n/a	

Jonckheere-Terpstra Step-Down Test

Control vs	Conc-µg ai/L	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	0	1.64	2	Asymp	0.7389	Non-Significant Effect
	6.8	0.555	1.64	3	Asymp	0.7389	Non-Significant Effect
	12	-0.059	1.64	3	Asymp	0.7389	Non-Significant Effect
	26	-0.587	1.64	3	Asymp	0.7389	Non-Significant Effect
	50	-0.64	1.64	3	Asymp	0.7389	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0123551	0.0024710	5	0.291	0.9112	Non-Significant Effect
Error	0.144167	0.0084804	17			
Total	0.156522		22			

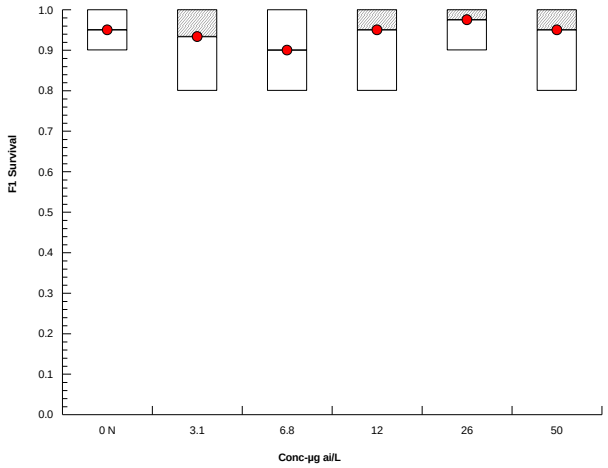
ANOVA Assumptions Tests

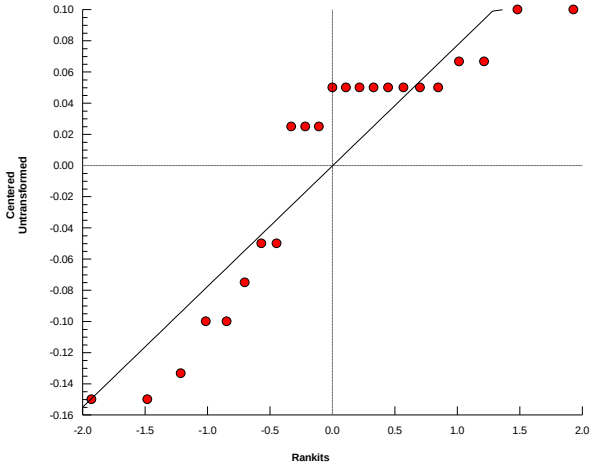
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	2.82	15.1	0.7280	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.844	0.88	0.0021	Non-Normal Distribution

F1 Survival Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.950	0.858	1.000	0.950	0.900	1.000	0.029	6.08%	0.00%
3.1		3	0.933	0.646	1.000	1.000	0.800	1.000	0.067	12.37%	1.75%
6.8		4	0.900	0.716	1.000	0.900	0.800	1.000	0.058	12.83%	5.26%
12		4	0.950	0.791	1.000	1.000	0.800	1.000	0.050	10.53%	0.00%
26		4	0.975	0.895	1.000	1.000	0.900	1.000	0.025	5.13%	-2.63%
50		4	0.950	0.791	1.000	1.000	0.800	1.000	0.050	10.53%	0.00%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) — **Smithers Viscient**

Analysis ID:	17-5040-8922	**Endpoint:**	n Offpspring Per Female	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	50	>50	n/a		44.68%

Dunnett Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	0.0385	2.41	6.26	6	CDF	0.8217	Non-Significant Effect
	6.8	0.308	2.41	6.26	6	CDF	0.7272	Non-Significant Effect
	12	-0.077	2.41	6.26	6	CDF	0.8622	Non-Significant Effect
	26	1.35	2.41	6.26	6	CDF	0.2806	Non-Significant Effect
	50	0.75	2.41	6.26	6	CDF	0.5350	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	41.755	8.351	5	0.618	0.6876	Non-Significant Effect
Error	243.15	13.5083	18			
Total	284.905		23			

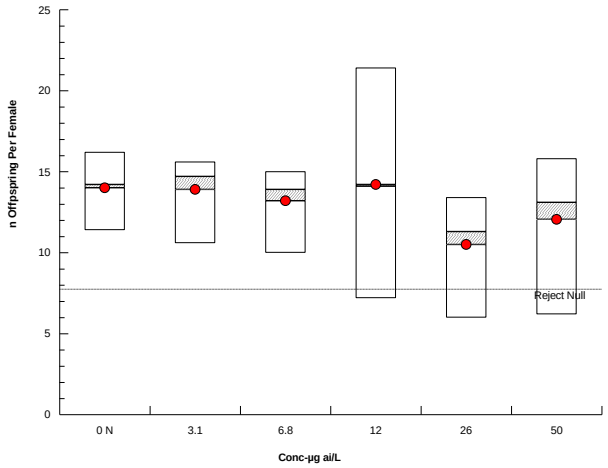
ANOVA Assumptions Tests

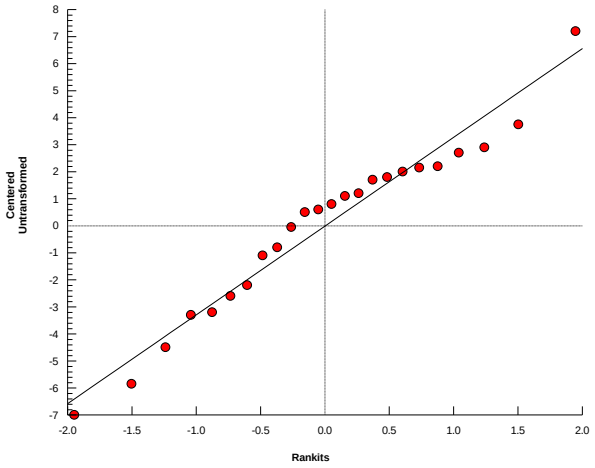
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	5.09	15.1	0.4047	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.965	0.884	0.5456	Normal Distribution

n Offpspring Per Female Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	14	10.6	17.4	14.2	11.4	16.2	1.07	15.25%	0.00%
3.1		4	13.9	10.3	17.5	14.7	10.6	15.6	1.13	16.22%	0.71%
6.8		4	13.2	9.7	16.7	13.9	10	15	1.1	16.64%	5.71%
12		4	14.2	4.58	23.8	14.1	7.2	21.4	3.02	42.58%	-1.43%
26		4	10.5	4.9	16.1	11.3	6	13.4	1.76	33.52%	25.00%
50		4	12	5.37	18.7	13.1	6.2	15.8	2.1	34.85%	13.93%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) **Smithers Viscient**

Analysis ID:	10-7456-4926	**Endpoint:**	n Offpspring Per Female	**CETIS Version:**	CETISv1.9.2
Analyzed:	30 Aug-18 18:54	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	50	>50	n/a		34.66%

Williams Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	0.0385	1.73	4.51	6	CDF	>0.05	Non-Significant Effect
	6.8	0.308	1.82	4.72	6	CDF	>0.05	Non-Significant Effect
	12	0.115	1.85	4.79	6	CDF	>0.05	Non-Significant Effect
	26	1.35	1.86	4.83	6	CDF	>0.05	Non-Significant Effect
	50	1.05	1.87	4.85	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	41.755	8.351	5	0.618	0.6876	Non-Significant Effect
Error	243.15	13.5083	18			
Total	284.905		23			

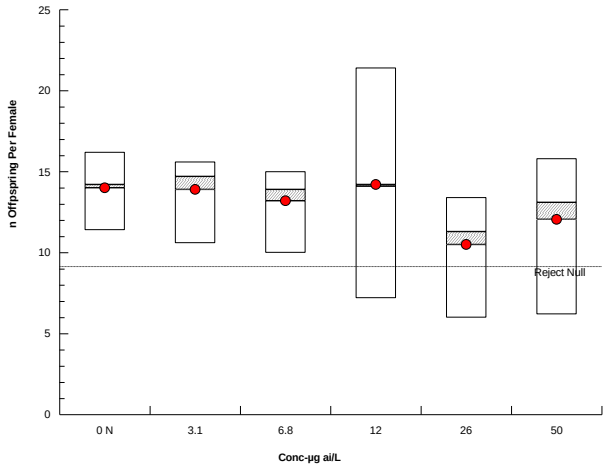
ANOVA Assumptions Tests

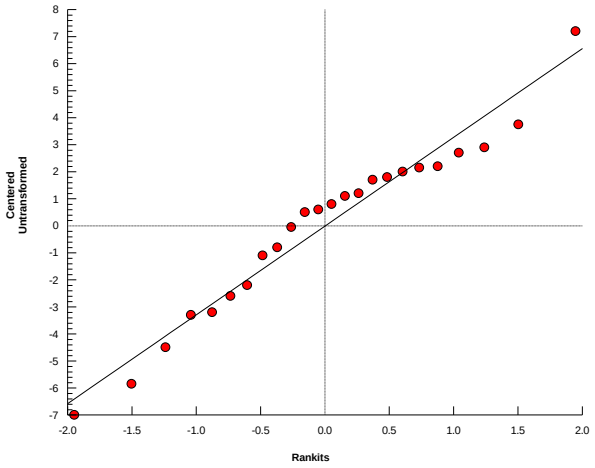
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	5.09	15.1	0.4047	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.965	0.884	0.5456	Normal Distribution

n Offpspring Per Female Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	14	10.6	17.4	14.2	11.4	16.2	1.07	15.25%	0.00%
3.1		4	13.9	10.3	17.5	14.7	10.6	15.6	1.13	16.22%	0.71%
6.8		4	13.2	9.7	16.7	13.9	10	15	1.1	16.64%	5.71%
12		4	14.2	4.58	23.8	14.1	7.2	21.4	3.02	42.58%	-1.43%
26		4	10.5	4.9	16.1	11.3	6	13.4	1.76	33.52%	25.00%
50		4	12	5.37	18.7	13.1	6.2	15.8	2.1	34.85%	13.93%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) — **Smithers Viscient**

Analysis ID:	21-1636-8021	**Endpoint:**	Time to First Brood	**CETIS Version:**	CETISv1.9.5
Analyzed:	30 Oct-18 13:28	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	50	>50	n/a		9.15%

Dunnett Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	-1.36	2.41	1.77	6	CDF	0.9937	Non-Significant Effect
	6.8	-1.29	2.41	1.77	6	CDF	0.9924	Non-Significant Effect
	12	-1.33	2.41	1.77	6	CDF	0.9931	Non-Significant Effect
	26	-1.83	2.41	1.77	6	CDF	0.9985	Non-Significant Effect
	50	-1.65	2.41	1.77	6	CDF	0.9974	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.50802	0.901604	5	0.833	0.5433	Non-Significant Effect
Error	19.4894	1.08274	18			
Total	23.9974		23			

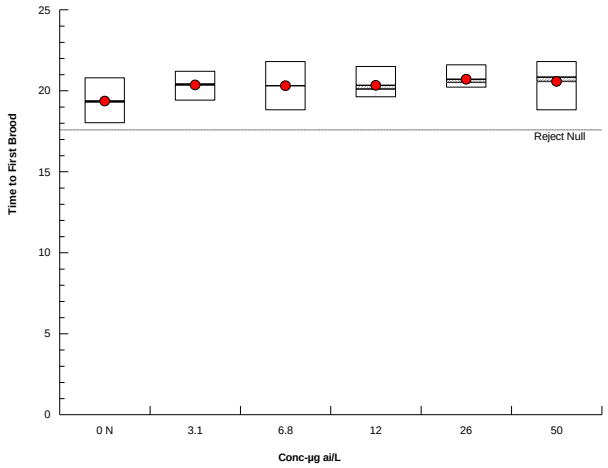
ANOVA Assumptions Tests

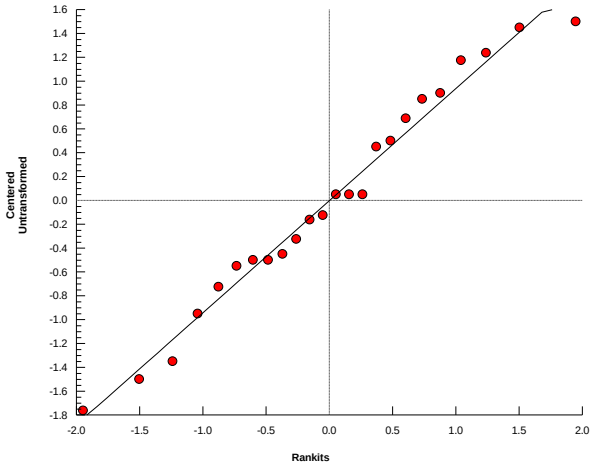
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	2.4	15.1	0.7914	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.97	0.884	0.6781	Normal Distribution

Time to First Brood Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	19.3	17.4	21.3	19.3	18	20.8	0.608	6.28%	0.00%
3.1		4	20.3	19.2	21.5	20.4	19.4	21.2	0.369	3.62%	-5.17%
6.8		4	20.3	18.2	22.4	20.3	18.8	21.8	0.645	6.36%	-4.91%
12		4	20.3	19	21.6	20.1	19.6	21.5	0.411	4.04%	-5.04%
26		4	20.7	19.7	21.7	20.5	20.2	21.6	0.325	3.14%	-6.98%
50		4	20.6	18.5	22.6	20.8	18.8	21.8	0.654	6.36%	-6.27%

Graphics





OPPTS 850.1350 Chronic Invert (Mysid) — **Smithers Viscient**

Analysis ID:	07-8833-4348	**Endpoint:**	Time to First Brood	**CETIS Version:**	CETISv1.9.5
Analyzed:	30 Oct-18 13:33	**Analysis:**	Parametric-Control vs Ord.Treatments	**Status Level:**	1
Batch ID:	16-2502-4673	**Test Type:**	Chronic Mysid (28-d)	**Analyst:**	
Start Date:	27 Apr-16	**Protocol:**	OPPTS 850.1350 Chronic Invert (Mysid Life	**Diluent:**	Natural seawater & well water
Ending Date:	25 May-16	**Species:**	Americamysis bahia	**Brine:**	
Test Length:	28d 0h	**Taxon:**		**Source:**	Smithers Viscient **Age:** <24

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	50	>50	n/a		7.10%

Williams Multiple Comparison Test

Control vs	Conc-µg ai/L	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control	3.1	-1.36	1.73	1.28	6	CDF	>0.05	Non-Significant Effect
	6.8	-1.29	1.82	1.34	6	CDF	>0.05	Non-Significant Effect
	12	-1.31	1.85	1.36	6	CDF	>0.05	Non-Significant Effect
	26	-1.45	1.86	1.37	6	CDF	>0.05	Non-Significant Effect
	50	-1.49	1.87	1.37	6	CDF	>0.05	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.50802	0.901604	5	0.833	0.5433	Non-Significant Effect
Error	19.4894	1.08274	18			
Total	23.9974		23			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	2.4	15.1	0.7914	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.97	0.884	0.6781	Normal Distribution

Time to First Brood Summary

Conc-µg ai/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	19.3	17.4	21.3	19.3	18	20.8	0.608	6.28%	0.00%
3.1		4	20.3	19.2	21.5	20.4	19.4	21.2	0.369	3.62%	-5.17%
6.8		4	20.3	18.2	22.4	20.3	18.8	21.8	0.645	6.36%	-4.91%
12		4	20.3	19	21.6	20.1	19.6	21.5	0.411	4.04%	-5.04%
26		4	20.7	19.7	21.7	20.5	20.2	21.6	0.325	3.14%	-6.98%
50		4	20.6	18.5	22.6	20.8	18.8	21.8	0.654	6.36%	-6.27%

Graphics